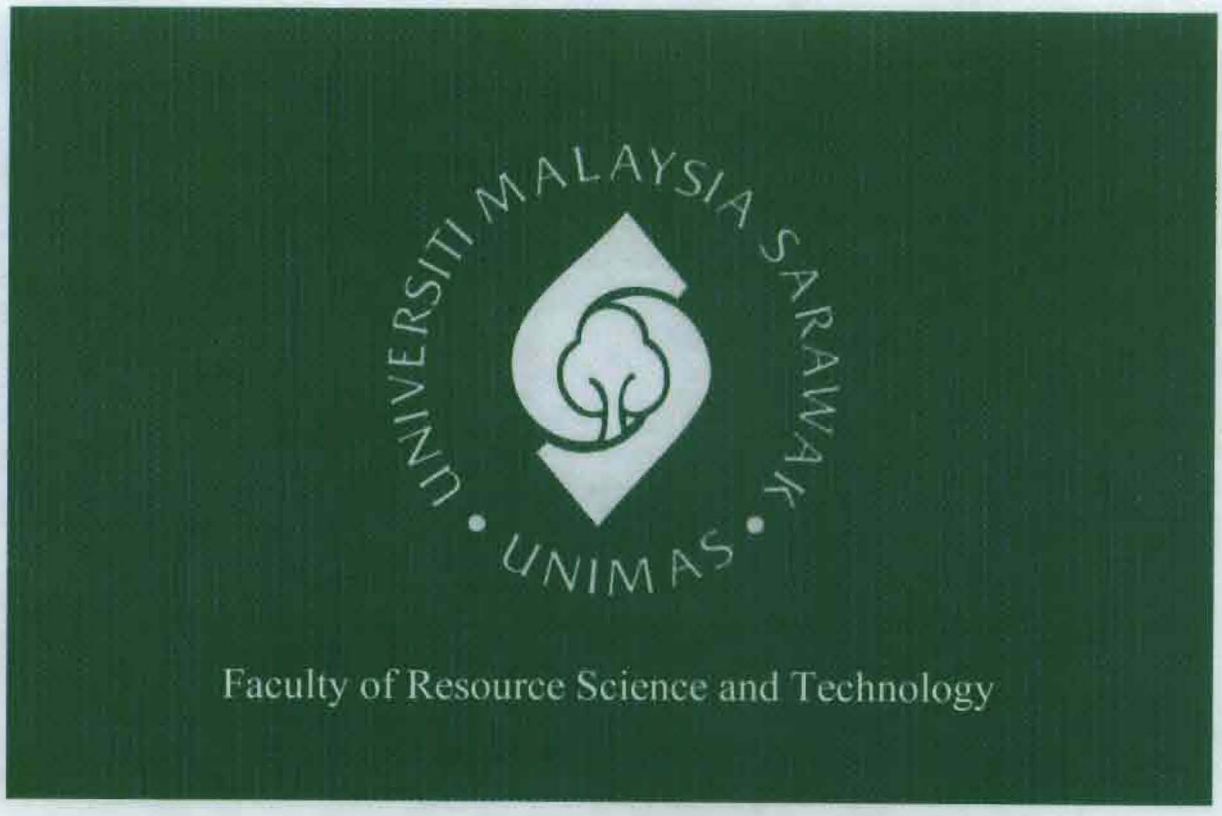

DEVELOPMENT OF PURIFICATION AND CRYSTALLIZATION OF YAM SUGAR

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Development of Purification and Crystallization of Yam Sugar

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Declaration

I hereby declare that no portion of the work referred in this project has be submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

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List of Abbreviations

cm	Centimeter
DC	Dried Cell
DCW	Dried Cell Weighted
DE	Dextrose equivalent
g	Gram
g/L	Gram per liter
HYS	Hydrolyzed Yam Starch
Kg	Kilogram
KNU	Kilo Novo Units
L	Liter
M	Meter
Mg	Miligram
ml	Mililiter
Mm	Milimeter
NaOH	Sodium Hydroxide
nm	Nanometer
PAC	Powdered Activated Carbon
PYS	Purified Yam Sugar
rpm	Round per minute
THS	Total Hydrolysable Starch
°C	Degree Celsius
µl	Microliter

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Abstract

This project is to produce crystal yam (*Colocasia esculenta* (L.) Schott.) sugar from. The main objective of this study is to compare the glucose obtained from two different varieties of yam (variety A and variety B). The different varieties of yam were obtained from both market (variety A) and field (variety B) independently, and the starch was hydrolyzed into glucose. Enzymatic hydrolysis was completed in 2 stages which are liquefaction (using Termamyl-120L) and saccharification (using AMG enzyme). The crystallized glucose was produced by purifying the HYS using PAC by either gravitational forces or peristaltic pump followed with crystallizing the PYS using either boiling, oven drying, or refrigerator cooling. The result on purifying of glucose show that, higher glucose recovery rate was able to be achieved by using gravitational force at glucose recovery rate of 80.8% as compared to using of peristaltic pump at glucose recovery rate of 71.7%. In the crystallization process, only refrigerator drying showed promising result in attempt to crystallize the glucose by crystallizing within 8 days. The glucose recovery rate of variety A was higher as compared to variety B with 95.72% and 61.83%, respectively. There was about 251.13g of glucose hydrolyzed from 1kg of variety A and variety B only able to produce 129.50g of glucose. The total mass of fresh yam required in producing 1kg of sugar for variety A and variety B was 12.29kg and 19.04kg, respectively.

Keyword: Yam, hydrolyzed yam starch (HYS), purified yam sugar (PYS), purification, glucose

Abstrak

*Projek ini adalah untuk menghasilkan kristal gula dari keladi (*Colocasia esculenta *(L.)* Schott*). Objektif utama kajian ini adalah untuk membandingkan glukosa yang diperolehi daripada dua variasi keladi (variasi A dan variasi B). Jenis variasi keladi telah diperolehi daripada pasaran (variasi A) dan ladang (variasi B), dan kanji telah dihidrolisiskan untuk menjadkani glukosa. Hidrolisis enzim keladi dibahagikan kepada 2 peringkat, iaitu pencairan (menggunakan Termamyl-120L) dan sakarifikasi (menggunakan enzim AMG). Glukosa yang jernih telah diperolehi melalui penulenan HYS dengan menggunakan PAC sama ada melaluih daya graviti ataupun pam peristalsis diikuti dengan kristalisasi PYS yang menggunakan sama ada pemanasan, pengeringan oven, ataupun penyejukan peti sejuk. Kadar pemulihan glukosa yang dicapai melalui penulenan menggunakan daya graviti menunjukkan kadar pemulihan glukosa yang lebih tinggi iaitu pada 80.8% berbanding dengan penulenan menggunakan pam peristalsis yang menunjukkan kadar pemulihan glukosa pada 71.7%. Dalam proses kristalisasi, hanya pengeringan sejuk menunjukkan pencapaian dalam usaha untuk mengkristalisasikan glukosa dengan menggunakan masa selama 8 hari. Kadar pemulihan glukosa daripada kanji menunjukkan bahawa variasi A dapat mencapai kadar pemulihan yang lebih tinggi berbanding dengan variasi B dengan 95,72% dan 61,83% masing-masing. Terdapat kira-kira 251.13g glukosa boleh dihidrolisiskan dari 1kg variasi A dan variasi B hanya mampu menghasilkan 129.50g glukosa. Jumlah jisim keladi segar yang diperlukan dalam menghasilkan 1kg gula daripada variasi A dan variasi B adalah 12.29kg dan 19.04kg masing-masing.*

Kata kunci: Keladi, keladi kanji dihidrolisiskan (HYS), gula keladi dipenulen (PYS), penulenan, glukosa

CHAPTER 1: INTRODUCTION

Yam is a tuberous plant which is consumed by human with several different species available locally. In Malaysia, yam is known as "keladi" and is referred to as *Colocasia esculenta* (L.) Schott. However, in countries outside Malaysia, *C. esculenta* is also known as taro, cocoyam, Talo-Tonga, Gabi, and Dalo (Onwueme, 1999; Mweta, *et al.,* 2008).

Yam is able to grow in saline soil which enables it to be planted across coastal sites, on soils that are not suitable for growth of most plant species (Onwueme, 1999; Haque, 2006). This indicated the potential of yam to be grown on unfertile and marginal land for extra income to the farmers.

Sugar produced from starch can be used for consumption, and for production of lactic acids and ethanol. Lactic acid is a precious compound in the cosmetics, pharmaceutical and food industries (Bujang, 2010). Lactic acid is an expensive chemical substance (US$50/L) compared to sugar (US$0.70/kg) and thus, fermentation of sugar into lactic acid will fetch a higher price with longer marginal benefit. The use of yam as bio-fuel is likely to cause food shortage and increase in the price of yam. However, it is likely to help in overcoming poverty of the farmer and the economy of the country (Worldwatch Institute, 2007).

C. esculenta comes from the family *Araceae* and has a large leaf area and remarkable look (Moon *et al.*, 2010). According to Onwueme (1999), there are several different varieties of *C. esculenta* found in Malaysia including Chinese yam "keladi China", and Penang yam "keladi Pinang". This tuber plant contained high percentage of dry starch basis at about 70 to 80% (Payne *et al.*, 1938; Tu *et al.*, 1979) and is widely distributed across wetland and dry land environment of tropical regions including Hawaii and Samoa (Jane *et al.,* 1992). Although this

plant species is known to originate from Indo-Malayan region (Lakhanpaul *et al.*, 2003), it is not widely distributed across Malaysia.

Yam possesses a problem in starch extraction due to the presence of non-starch polysaccharides (NSP) that lowered the total yield from starch extraction (Daiuto *et al.*, 2005). NSP are polysaccharides that do not include starch, such as cellulose and pectin.

Sugar is produced from several steps, which are pulverization of yam tuber followed by adding 0.03M ammonia to increase the starch yield (Moorthy, 1991). The starch was then hydrolyzed into glucose by enzymes which are α-amylase and amyloglucosidase (Fujii *et al.*, 1988). Sugar is then purified using activated carbon multi-filtration. The purified sugar is then crystallized by using spray dried methods to obtain sugar crystal.

The main objectives of this project are to:

1. Study the different physical characteristics in hydrolysis process from two varieties of yam.
2. Study the purification methods for purifying yam sugar.
3. Study the crystallization methods for crystallizing yam sugar.
4. Compare the glucose recovery between two varieties of yam.
5. Study the starch content between the two varieties of yam.

CHAPTER 2: LITERETURE REVIEW

2.1 Yam

2.1.1 Biological and Physiological Characteristics of Yam

Yam is an edible tuber plant from family *Araceas*; the shape of the leaves assembled a shield structure as shown in **Figure 1**. It is able to grow about 1 to 2 m tall (Deo *et al.*, 2009). Even though it originates from Indo-Malayan (Lakhanpaul *et al.*, 2003), yam is now widely distributed and has become staple food among developing countries at Asia-Pacific and Africa (Tumuhimbise *et al.*, 2009).

Figure 1: Leave shape of *Colocasia esculenta* (Retrieved on October 30th, 2012, from http://images.harc.edu/Sites/GalvBayInvasives/Species/ColocasiaEsculenta.jpg)

The harvesting periods of yam in Malaysia can be divided into two categories based on variety which are short season (4 to 6 months) examples are Keladi Pinang and Keladi

Tongsan and the longer-season (9 to 12 months) examples are Keladi China and Batang Hitam (Onwueme, 1999)

Yam is able to grow on certain harsh environment where other plants are unable to tolerate including wetland, saline soil, and shady environment (Onwueme, 1999). The ability of yam to grow on wetland was further supported by Bussell and Bonin (2010), which indicated a better growth rate of yam at high watering level. Japan and Egypt had grown yam on saline soil that had low fertility (Onwueme, 1999). Yam is shown to grow well at wetland as shown in **Figure 2** below.

Figure 2: Growth of yam on wetland (Retrieved on October 30th, 2012, from http://homeguides.sfgate.com/DM-Resize/photos.demandstudios.com/getty/article/251/98/78029493.jpg?w=600&h=600&keep_ratio=1)

2.1.2 Tuber Composition

Yam has many different varieties in the market **Figure 3 and Figure 4** shown was some of the varieties of yam. The quantity of copper and iron affects the coloring of tuber and its flour, where high copper content causes shade of brown of the tuber and intermediate iron content

causes darkening of the flour (Njoku and Ohia, 2007). According to Sefa-Dedeh and Agyir-Sackey (2001), the chemical composition of tuber differs within the parts. This indicates, nutrients are well organized in the tuber of the yam. Generally, the fresh weight of yam is mainly consists of moisture (63 to 85%), carbohydrate (13 to 29%), protein (1.4 to 3.0%), fat (0.16 to 0.36%), crude fibre (0.60 to 1.18%), and 0.60 to 1.30% of ash (Onwueme, 1999). According to Tattiyakul *et al.* (2006), a high average rainfall contributed to high carbohydrate content in the tuber. Yam also contained oxalic acid which causes gallstone deposition over long period of consumption (Tattiyakul *et al.*, 2006). However, according to Iwuoha and Kalu (1994), effects of calcium oxalate is reduced via boiling.

Figure 3. Tuber of yam. (Retrieved on October 30th, 2012, from http://upload.wikimedia.org/wikipedia/commons/thumb/6/66/Colocasia_esculenta_P1190432.jpg/220px-Colocasia_esculenta_P1190432.jpg)

Figure 4: Tuber of yam. (Retrieved on October 30th, 2012, from http://upload.wikimedia.org/wikipedia/commons/c/c2/Colocasia_esculenta_dsc07801.jpg)

2.1.3 Characteristics of Starch from Yam

C. esculenta is an edible tuber plant that has non-starch polysaccharides (NSP). NSP reduced the quality of starch upon extraction (Daiuto *et al.,* 2005). There are about 70-80% of dry starch basis in yam. The starch of yam occupied 77.9% of the total carbohydrate in the tuber (Onwueme, 1999). The starch of yam is stored in the starch granules, with small irregular and polygonal shapes (Tattiyakul *et al.,* 2006). The amylose content in starch ranging from 16.3% to 22% (Srichuwong *et al.*, 2005; Jane *et al.*, 1992). Meanwhile, the branch chain length of amylopectin ranged from 16.9 to 18.4 degree of polymerization (DP) for the short branches and 37.2 to 40.5 DP for the long branches (Jane *et al.*, 1992). Based on the study conducted by Jane *et. al.* (1992), all starches from different varieties of yam have an A-type X-ray

diffraction pattern (Tattiyakul *et al.*, 2006). The starch of yam had a gelatinization onset temperature ranging from 69.1 to 74°C (Jane *et al.*, 1992).

2.2 Enzymatic Hydrolysis of Starch

Starch is able to be hydrolyzed by amylase by breaking the α-1,4-glycosidic bond of amylose-amylopectin (Aiyer, 2006). Studied conducted by Griffin and Fogarty (1973) classified amylase's enzymatic activities into 6 categories according to the hydrolytic site of the enzyme on the bond of polysaccharide:

1. Hydrolyzed of α-1,4-glycosidic bond; bypass α-1,6-glycosidic bond (e.g.: α-amylase)
2. Hydrolyzed of α-1,4-glycosidic bond; cannot bypass α-1,6-glycosidic bond (e.g.: β-amylase)
3. Hydrolyzed of both α-1,4-glycosidic and α-1,6-glycosidic bond (e.g.: glucoamylase)
4. Hydrolyzed α-1,6-glycosidic bond (e.g.: pullulanase)
5. Hydrolyzed amylose and amylopectin of α-1,4-glycosidic bond from short chain of oligosaccharides that produced by other enzymes (e.g.: α-glucosidase)
6. Hydrolyzed starch into non-reducing cyclic D-glucosyl polymers (e.g.: *Bacillus macerans* amylase)

2.3 Liquefaction and Saccharification

Liquefaction is a process where starch is hydrolyzed into short dextrin chain that required prior gelatinization of the starch to increase the solubility of amylose in the starch granule (Crabb and Mitchinson, 1997). The enzyme used for liquefaction which is α-amylase are able to tolerate at high temperature as high as 105°C, but due to the inability of the enzyme to function at pH5.9 had caused liquefaction process to be controlled between pH 5.8 to pH 6.5. Meanwhile, saccharification is a process where required the use of two different enzyme which are glucoamylase and pullulanase that functioned well at acidic pH. This in term had caused pH to be reduced in between pH4.2 to pH4.5 at 60°C (Crabb and Mitchinson, 1997).

2.4 Activated Carbon

Activated carbon is a carbonaceous substance that had micropores in slit-shaped (Karanfil and Kilduff, 1999). The porous size of active carbon can be grouped into three categories which are:

1. Narrow and wide microporosity (<2.0 nm)
2. Mesoporosity (2.0-50 nm)
3. Macroporosity (>50 nm)

Besides the porosity, chemistry of surfaces on the pore also plays an important role in eliminating the unwanted substances. Impurities also removed by the van der Waals forces of pores in active carbon. Overall, the size, shape, chemistry, and properties of pores had their own role in affecting the purification ability of active carbon. Liquid solution is able to be

purified by either Granular Activated Carbon (GAC) or Powdered Activated Carbon (PAC) in liquid-phase application. The liquid phase application can be divided into 2 types:

1. Removal of impurities from solution
2. Recovery of solute from solution

(Source: Marsh and Rodriguez-Reinoso, 2006)

Active carbon is a good material used in purification as it is able to be reused, reactivated or regenerated (Bujang, 2010).

2.5 Sugar

The sugar (glucose) can be obtained from hydrolysis of polysaccharides including cellulose and starch. High glucose content rate had been gained from starch with more than 99% dextrose equivalent (Booty and Bujang, 2009) compared to glucose that recovery from cellulose which is lower than 50% (Bujang, *pers. comm.*). Glucose produced through enzyme hydrolysis is able to be applied either directly as food source or indirectly by converting into ethanol, lactic acids or other pharmaceutical products (Bujang, 2010).

2.5.1 Direct Application

Sugar consumption in Malaysia is mainly come from sugar cane that have high annual production rate but low sugar recovery rate (Bujang, 2010). According to Booty and Bujang (2009), the sugar consumption rate in Malaysia had increased tremendously due to increase of food processing industry with 50 kg (raw equivalent) on per caput basis. This causes increased

in sugar importation from other countries and thus led sugar to become the largest agricultural imports in Malaysia (FAO, 1997).

2.5.2 Utilization of Sugar on Substitute for Production of Biofuel

The ever increase in price and non-renewable of petroleum had forced on finding alternative ways on powering human development. Ever since, biofuel, especially fermentation of bio-ethanol from starch or sugar rich biomass had arise and showed promising future in replacement of petroleum (IEA Energy Technology Essential, 2007; Crocker and Andrews, 2010). This was further supported by Nguyen *et al.* (2006), which indicated the potential of bio-ethanol to replace petroleum by improving the yield and market development of tuber plant. Besides that, bio-ethanol are also more environmental friendly comparing to fossil fuel, which produced less CO_2 emission as high as 90% reduction rate (IEA Energy Technology Essential, 2007). The environmental friendly feature of bio-ethanol was further concreted by using of rotten tuber plant in production of high bio-ethanol concentration as compared to theoretical yield (Liimatainen *et al.*, 2004; Ramesh *et al.*, 2010).

2.5.3 Fermentation of Sugar into Industry Lactic Acids

Since lactic acids played an important roles in the industry of food, chemical, cosmetic, preservatives and pharmacy, as well as in synthesis of polylactic acid (PLA) used for production of biodegradable plastic (Bujang *et al.*, 2001; Bujang, 2010). It caused a high value of lactic acids as compared to glucose and starch (Bujang, 2010). This can helped in

increasing the marginal benefits from the crops. Lactic acids have two types of isomeric properties, namely D-lactic acid and L-lactic acid. Only L-lactic acid are able to be fully assimilate by human body, which are fermentable by *Lactococcus lactis* IO-1 (Bujang, 2010). In view of the fact that *L. lactus* IO-1 only produce L-lactic acids, this enables decrease in the cost of production that yields higher marginal benefits. At the mean time, a study conducted by Bujang *et al.* (2000), had further enhanced lactic acids fermentation rate from glucose by studying the pH of *L. lactis* IO-1 that pointed out better lactic acid production rate at uncontrolled pH. Production of L-lactic acids from fermentation of glucose can be considered as an environmental friendly and very productive, because there are no CO_2 produced across the fermentation process (Bujang *et al.,* 2000) and each 1 mol of glucose can yield 2 mol of lactic acids (Bujang *et al.*, 2001).

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

3.1.1 Yam Sample

Fresh yam samples were obtained for this project, on two varieties, based on the color of the de-skinned tubers. The so-called variety A (from a market at Batu Tujuh) had a purple color strip and purple color dot that could be observed from the white tuber, and it priced at RM11.00/kg. Another type, name as variety B (from No 10, Batu 14 ½, Outer Ring, Jalan Kuching/Serian, 93280, Kuching) had a creamy white color of dot that could be observed from the white tuber, and it priced at RM10.00/kg. Both varieties (**Figure 5**) were used and analyzed for production of sugar in this project.

In liquefaction, the starch suspension in water was adjusted to pH 6 to 6.5 by addition of 1M of sodium hydroxide, NaOH which is the optimum condition for α-amylase. According to Crabb and Mitchinson (1997), gelatinizing the starch by heating over 100°C for a few minutes was able to remove all lipid-amylose complexes. Following that, 0.5µl of Termamyl (per gram of starch). The starch slurry was maintained at the temperature of 80 to 90°C for 1 to 2 hours.

Next, the pH was reduced to a pH4 to 4.5 and temperature was lowered to 60°C for the preparation of saccharification. After the pH and temperature was adjusted accordingly, 0.6 µl Dextrozyme (per gram of starch) was added to the HYS to hydrolyze the starch slurry into reducing sugar, glucose. The slurry is maintained at the described condition for 24 hours. The total yield of glucose from hydrolysis of starch slurry was calculated by percentage of glucose recovery.

3.2.4 Purification of Yam Sugar

3.2.4.1 Filtration on PAC by Gravity

A 5 cm diameter column was prepared and filled with 5 g PAC using glass wool (5 g) to block the lower end, as shown in **Figure 8**. The column was filled with 100 ml HYS and filtered under gravity. HYS was centrifuged (9000 rpm, 30 min) to obtain liquid yam sugar, free from solids but still brownish in color as shown in **Figure 9** before it was purified in the column.